Study Plan for Assessing Growth of *Enterococcus* in Storm Drains
County of San Diego Municipal Copermittees

Background

Urban runoff is a major non-point source of fecal indicator bacteria (FIB) to California's coastal waters. Elevated levels of FIB (total coliforms, fecal coliforms and *Enterococcus*), commonly occur in urban runoff conveyed to beaches by creeks and storm drains. The presence of these organisms is considered indicative of fecal contamination and potential risk for adverse health effects in swimmers and bathers, and as such, is the basis for implementing of bacterial TMDL plans.

Although FIB are always found in fecal material, they also occur in environmental settings where no obvious source of fecal contamination is present. This is because many FIB are capable of survival outside the digestive tracts of animals. For example, some species of *Enterococcus* are known to be associated with decaying plant material. Further, recent studies have shown that *Enterococcus* is able to persist and even grow in beach sand, where it may enter the water column via water runoff from land or through tidal and wave action.

In aquatic environments such as storm drains, *Enterococcus* bacteria may colonize and grow on surfaces by forming biofilm. Biofilm is a matrix formed when bacterial cells secrete substances that allow them to adhere to and grow on a surface. Once established, biofilms develop into a complex matrix containing diverse communities of bacteria. When biofilm containing enterococci forms on drain surfaces, bacteria may be sloughed off into the water by increased flow velocity or by scouring when debris move through the drain. Sloughed pieces of biofilm and bacteria may then be transported downstream where the bacteria colonize a new surface and continue their cycle of growth, or remain suspended in water discharged to beaches where they may impact water quality. This raises the possibility that bacterial growth within storm drains may be at least partly responsible for elevated concentrations of enterococci observed in urban runoff impacting beach water quality.

Study Objective

Biofilms in storm drains represent a potential reservoir of enterococci that may be dispersed to water bodies and lead to false conclusions regarding the presence of fecal pollution and human health risks. The purpose of this study is to assess storm drains as a potential source of *Enterococcus* bacteria to San Diego's marine coastal waters during dry weather.

The goals of this study are to:

1. Determine if biofilms that form on the surfaces of storm drains support the growth of enterococci.
2. Determine if enterococci growing on drain surfaces may be a source of bacteria to beach water and sand.
3. Determine if enterococci growing on drain surfaces may be differentiated from those of fecal origin

Sampling Period

Sampling will be conducted for up to a 12 week period during the dry season.

Study Locations

Two locations in San Diego County were selected as study sites: EH 420, Moonlight Beach, City of Encinitas and CSD037, City of San Diego.

The locations were selected based on meeting the following criteria:

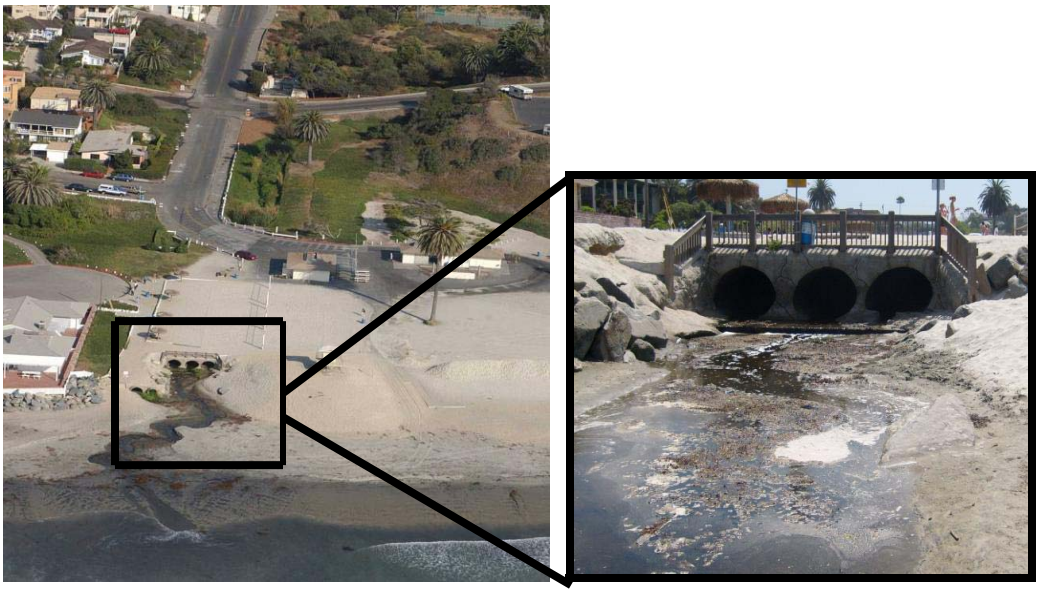
1. Safe access
2. The storm drain has nuisance flow
3. Storm drain is underground (with manhole access)
4. The location is above the tidal prism
5. The nuisance flow reaches the beach or discharges directly into the ocean

The study sites were recommended by the Copermittees' Coastal Storm Drain Monitoring Workgroup who considered sites with elevated historical enterococci counts in their analysis.

EH 420, Moonlight Beach, City of Encinitas

This site has characteristics of both urban and natural drainages. In August 2002, the City of Encinitas constructed an urban runoff treatment facility (UV Facility) along Cottonwood Creek to eliminate water quality exceedances and protect public health for those recreating at Moonlight Beach. The UV Facility is located approximately 230 yards upstream of Moonlight State Beach. It is sited adjacent to Cottonwood Creek at the southeast corner of Third and B Street. The UV Facility diverts 85% of the dry weather flows in Cottonwood Creek into an UV treatment system designed to inactivate bacteria and viruses in the creek water. A 15% by-pass was required by the San Diego Regional Water Quality Control Board to allow for biological connectivity. Bacterial removal efficiencies through the UV Facility were calculated at >99% based on 153 pairs of influent and effluent samples. Although the majority of water flowing through the drain is

sterilized, it feeds into a ponded area with ample water plants and decaying plant matter before entering the underground pipe that terminates on the beach.



CSDO37, City of San Diego

This storm drain has steady flow and appears to have a consistent algal layer in the bottom of the pipe and routine monitoring has revealed consistently elevated concentrations of *Enterococcus*. The largest counts (42,000 CFU/100ml) were obtained on July 9, 2008. From February 2008 through March 2009, the storm drain averaged 6,400 CFU/100 ml. Upstream at the manhole (corner of Midway Street/Chelsea Avenue), the drain is 8 ft deep with no apparent sediment or debris. The outfall discharges to a cobble beach that is accessible at all tides. It is not evident that the flow makes it to the receiving water on a consistent basis due to the cobble on the beach.

Study Design

The study will utilize multiple approaches for assessing growth of *Enterococcus* in storm drains. The primary method will involve using a series of fabricated concrete coupons to examine biofilm development. It is hypothesized that enterococci in runoff flows will attach to the coupons, develop biofilm and begin replicating. Thus, bacterial densities on coupon surfaces should increase over time. Multiple coupons will be placed in each drain and densities of enterococci on coupons will be measured at regular intervals by collecting individual coupons to assess growth.

Another method for assessing biofilm associated growth will involve extracting enterococci from natural materials (i.e., algae and sediments) already present inside of storm drains and at the outlets to beach sites. High densities of *Enterococcus* extracted from natural materials will be highly suggestive of bacterial regrowth.

Finally, *Enterococcus* and bacterial DNA isolated from coupons, algae and sediments will be compared with isolates and DNA from storm drain and beach water samples using advanced typing methods such as speciation and genetic analysis of isolates, and community fingerprinting of bacteria. Speciation will determine the proportions of different enterococcal species found in these samples and to assess transport of related or clonal populations of *Enterococcus* from storm drains to beach water. Community fingerprinting will assess the extent to which bacterial populations in dry weather flows discharged to the beach and in beach sand are related to those found on drain surfaces and organic materials.

Assessment of Enterococcal Biofilm Development

Concrete coupons measuring approx. 4” x 4” will be fabricated or cut from a pre-existing storm drain pipe. The coupons will be sterilized and placed into metal lath cages and securely fastened within storm drains. The coupons will be immersed in water generated from dry weather flows to promote biofilm formation. After a 2-week “incubation” period, two coupons will be collected and processed to enumerate enterococci extract bacterial DNA, leaving the remaining coupons in place. Subsequently, two coupons will be removed and tested on an approximately weekly basis.

Sample Collection

Samples will be collected by personnel from City of Encinitas (Moonlight Creek) and City of San Diego (CSDO37) and transported to the City of San Diego Laboratory for processing. On each sampling occasion, coupons will be taken from each cage, immersed gently into bottles containing phosphate buffer saline (PBS) solution and held on ice until transported to the laboratory for processing. Prior to removing the coupons, dry weather urban flows will be collected from upstream and downstream locations in

relation to the placement of the coupons within the drain and a beach water sample will be collected at the drain outlet. Plants and algae growing within the drain and sand at beach outlets will also be collected and analyzed for enterococci.

Extraction Methods

SCCWRP personnel will train personnel at the City of San Diego (CSD) laboratory on extracting enterococci from coupons and natural materials such as sand, algae and plant material. Samples for DNA extraction will be filtered on site or preserved by CSD technicians as time and workload permit or by SCCWRP personnel. Specific protocols will be provided by SCCWRP.

Briefly, coupons stored in PBS will be shaken gently to remove loosely attached bacteria. The elutriate will be poured off, shaken vigorously and filtered similarly to water samples. The coupons will then be rinsed with PBS and brushed vigorously (using clean toothbrushes) to remove adherent biofilm. The second elutriate will also be shaken and filtered as before. The concentrations of enterococci that are loosely attached will be compared to those that are tightly bound to coupon surfaces. Bacteria for DNA analysis will be captured by filtration and stored frozen until analyzed.

Procedures for extracting enterococci from beach sand will follow those developed by SCCWRP as part of the Bight '08 regional monitoring program. Extraction of enterococci and DNA from algae and plant material will be by shaking a pre-determined mass in sterile buffer.

Enterococci Isolation and Enumeration Methods

Enterococci in water samples and elutriates will be processed using EPA Method 1600, a membrane filtration method using mEI agar. Enterococci will be enumerated based on coupon surface area; 100 ml of water or per dry weight of sand or algae and plants. Samples will be tested in duplicate to assess reproducibility and to ensure accuracy of enterococci counts. A subset of colonies will be isolated from agar plates for speciation.

Enterococci Speciation Methods

Enterococci isolated from various sample types (i.e. water, coupons, sand, algae) will be enumerated, however speciation will be done only on samples collected at the beginning, middle and end of the study period. Initially, 50 isolates per sample will be identified to species level. Adjustments to the number of isolates selected for analysis to the species level may be made based on results form the initial samples.

Isolates that are presumptive for enterococci will be identified to species level using Vitek and additional tests such as pigment production, motility and carbohydrate utilization. SCCWRP will train local laboratories on species identification methods as needed. After the isolates have been speciated, they will be archived and a subset will be selected for genetic testing.

Community Fingerprinting

Bacterial DNA will be analyzed using TRFLP (terminal-Restiction Fragment Length Polymorphism). This method will produce a snapshot of the bacterial community from each water, coupon, sand, or organic material sample. This "community fingerprint" will allow comparison of the bacterial communities present and provide additional evidence toward determining the sources of bacteria to the receiving waters.

Additional Measurements

Water samples will be analyzed for additional physical and chemical parameters including:

1. temperature
2. turbidity
3. pH
4. conductivity
5. dissolved oxygen

Data Analysis

Regrowth will be evaluated using the following criteria:

1. Densities of enterococci recovered from coupons and natural samples (i.e. sediment, algae)
2. Increase in densities of enterococci on coupons over time
3. Presence of clonal phenotypes or genotypes of enterococci

Natural versus fecal sources of enterococci will be evaluated based on:

1. Percentage of plant associated species (*E. casseliflavus* and *E. mundtii*) and species commonly found in fecal and natural sources (*E. faecalis* and *E. faecium*)
2. DNA typing results comparing enterococci genotypes from humans and animals,
3. Bacterial community relatedness as demonstrated by TRFLP data

SCCWRP will collect and manage FIB data from the analysis of test samples. The data will be summarized in tables and the data will be analyzed. The Copermittees will

include the report in the San Diego County Municipal Copermittees 2009-2010 Urban Runoff Monitoring report that is due to the San Diego Regional Water Quality Control Board on January 31, 2011.